FEULGEN CYTOPHOTOMETRIC DETERMINATION OF NUCLEAR DNA IN SPECIES OF *CHARA* (CHAROPHYTA) FROM INDIA

Ruma PAL and Probir CHATTERJEE*

ABSTRACT – DNA contents of seven taxa of the genus *Chara* were measured from Feulgen stained 2C nuclei (metaphase) of the antheridial filament cells using cytophotometric method. The DNA content has been expressed in arbitrary units of relative absorbance. The DNA value obtained have been correlated with other cytological data like nuclear volume and chromatin length and from ■ analysis of these parameters a tentative phylogenetic scheme of evolution of these taxa has been presented. *C. corallina* showed the highest DNA value corresponding to its high chromosomes number, nuclear volume and chromatin length. *C. fibrosa* (n = 42) because of its smaller chromosomes size and nuclear volume has less DNA at metaphase. Among the four species with 28 chromosomes, *C. vulgaris* var. *inconnexa* f. *hippelliana* (n = 28) showed the least 2C DNA corresponding to its smaller nuclear volume and chromatin length. The highest DNA value of 28 chromosomes species studied was recorded in *C. zeylanica* var. & f. *zeylanica* which also showed a positive correlation with nuclear volume and chromatin length.

RÉSUMÉ – Les teneurs en ADN de sept taxons du genre *Chara* sont mesurées par la méthode cytophotométrique appliquée à des noyaux au niveau 2C (métaphase) colorés au Feulgen provenant de cellules de filaments anthéridiaux. Exprimée en unités arbitraires d'absorbance relative, la teneur en ADN ainsi obtenue est corrélée avec d'autres données cytologiques telles que volume nucléaire et longueur de la chromatine. A partir de l'analyse de ces paramètres, un essai de schéma phylogénétique de l'évolution de ces taxons est présenté. *Chara corallina* offre la teneur en ADN la plus élevée, ce qui correspond à son grand nombre de chromosomes, au volume nucléaire et à la longueur de la chromatine. Du fait de la dimension des chromosomes et de son volume nucléaire plus petits, *C. fibrosa* (n = 42) contient moins d'ADN à la métaphase. Parmi les quatre espèces à 28 chromosomes, *C. vulgaris* var. *inconnexa* f. *hippeliana* présente la teneur la plus faible en ADN (stade 2C) ce qui correspond à son volume nucléaire et sa longueur de chromatine plus faibles. La valeur la plus élevée en ADN chez les espèces à 28 chromosomes étudiées est relevée chez *C. zeylanica* var. et f. *zeylanica* qui montre également une corrélation positive avec le volume nucléaire et la longueur de la chromatine. (traduit par la rédaction)

KEY WORDS : *Chara*, Feulgen, Cytophotometry, DNA.

* Centre of Advanced Study, Department of Botany, University of Calcutta - 35, Ballygunge Circular Road, Calcutta - 700 019.

INTRODUCTION

Feulgen cytophotometry has been utilized in recent years as a tool for determining the DNA content of plant nuclei. In angiospermic plants such studies have been carried out in many genera (PRICE, 1976), but data with regard to lower plants particularly with algae are so far meagre. Feulgen cytophotometric determination of DNA in case of algae has mostly been utilized to determine the site of meiosis in cases where the counting of chromosomes presents difficulties. DNA content determination was carried out in case of green algae (Chlorophyta) such as *Acetabularia* (KOOP, 1975), *Fritschiella tuberosa* (HOPKINS, 1977) and *Pandorina* (COLEMAN, 1979), etc. In case of the green algal division Charophyta, there was a solitary report of Feulgen cytophotometry in case of *Chara zeylanica* by SHEN (1967). The present authors measured the DNA of 2C nuclei at metaphase of some species of *Nitella* (PAL and CHATTERJEE, 1986).

Besides feulgen cytophotometry, microfluorometric determination of DNA in case of algae has also been made in recent years (HULL et al., 1982) but not yet in Charophyta. Since there is a paucity of information with regard to DNA content of 2C nuclei in cases of *Chara* species, the present investigation was taken up.

MATERIALS AND METHODS

Seven species of *Chara* were investigated, which were collected from different parts of West Bengal, India. They were *C. corallina* Klein ex Willd. var. & f. *corallina*, *C. zeylanica* Klein ex Willd. var. & f. *zeylanica*, *C. setosa* Klein ex Willd. f. *setosa*, *C. globularis* Thuill. var. & f. *globularis*, *C. vulgaris* L. var. *in-*

Name of the species	Chromosome number (n)	Total chromatin length (μm)	Nuclear volume (μm3)	DNA content in arbitrary unit (2C level)
1. *C. vulgaris* var. *inconnexa* f. *hippelliana*	28	117.1	796.3	0.96 ± 0.01
2. *C. globularis* var. & f. *globularis*	28	128.3	943.6	1.01 ± 0.01
3. *C. setosa* f. *setosa*	28	130.0	992.4	0.99 ± 0.001
4. *C. braunii* f. *braunii*	14	74.2	161.1	0.72 ± 0.01
5. *C. corallina* var. & f. *corallina*	42	182.1	1323.8	1.21 ± 0.004
6. *C. fibrosa* var. & f. *fibrosa*	42	97.8	776.4	0.94 ± 0.002
7. *C. zeylanica* var. & f. *zeylanica*	28	132.2	1112.4	1.08 ± 0.004

Table I : Showing chromosome number, total chromatin length, nuclear volume and DNA content (at 2C level) in seven species of *Chara*.

connexa (TFA) R.D.W. f. *hippelliana*, *C. fibrosa* Ag. ex Bruz. var. & f. *fibrosa*, *C. braunii* Gm. f. *braunii*.

Plants were grown in soil water biphasic medium. For the methods employed, see PAL and CHATTERJEE (1986).

RESULTS

Of all the species studied, *C. corallina* (n = 42) having largest nuclei and highest chromatin length contained maximum amount of DNA at 2C level (vide Table I, Figs. 1 & 2). Though *C. fibrosa* had the same chromosome number of n = 42,

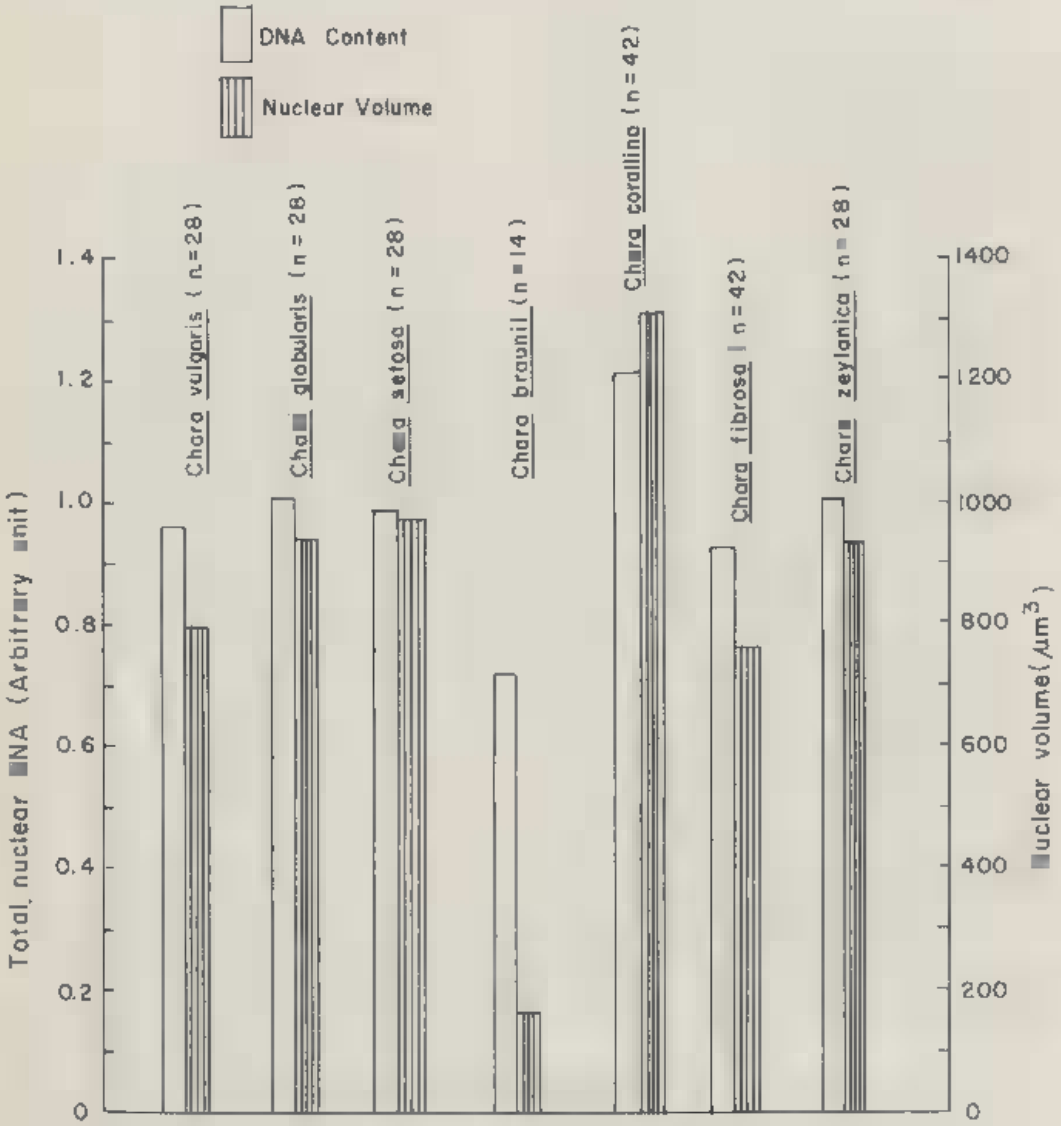


Fig. 1 — Histogram showing DNA content and nuclear volume in seven species of *Chara*.

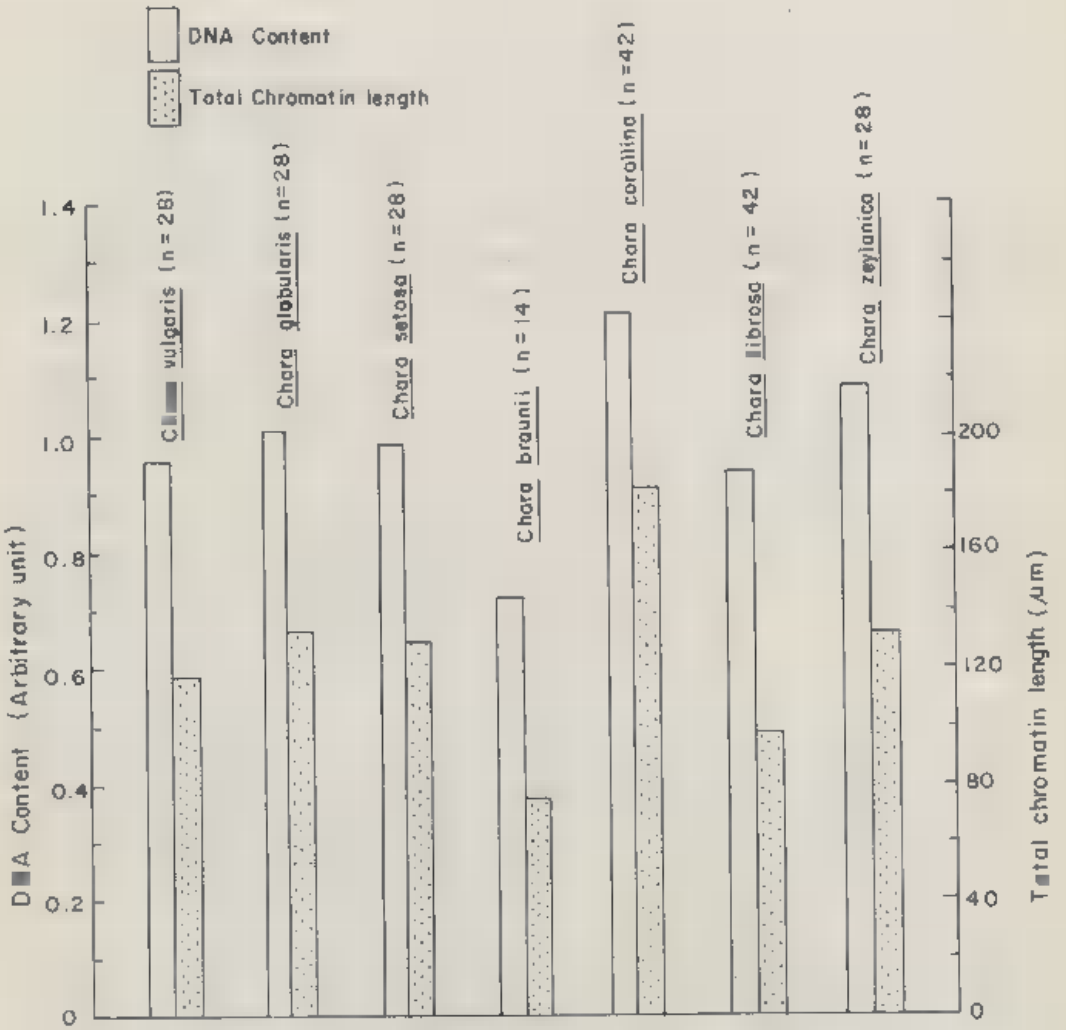


Fig. 2 – Histogram showing DNA content and total chromatin length in seven species of *Chara*.

its DNA value was much less than *C. corallina*, even less than other 28 chromosome species. The nuclear volume and the chromatin length of this species was also less than the other species.

Among the four species with 28 chromosomes, *C. vulgaris* had less DNA than the other three species, whose nuclear volume and chromatin length were also less. *C. zeylanica* showing maximum amount of DNA among the 28 chromosome species, also showed correspondingly higher nuclear volume and chromatin length. In the present study *C. braunii* (n = 14) showed the lowest DNA content.

DISCUSSION

It is evident from the results obtained that the variation in DNA content among the 7 species of *Chara* is not much significant which is nearly 1.5 to

2-fold. In case of *C. braunii* where the total chromatin length is nearly half of the 28 chromosome species, the nuclear volume of this species was about one-fifth than that of others, still the difference in DNA value is small. The reasons for this difference may be attributed to two facts :

1) All the species were growing in almost identical habitat and ecological condition, and 2) All the species were annual.

Similar results were obtained by STEBBINS (1950, 1966), BENNETT (1972) and others in cases of higher plants, who showed a correlation of total DNA content with their growth forms.

Study of DNA variation at the taxonomical level is highly important, as from these results the evolutionary tendency in DNA content may be drawn in some cases. In Characeae, the evolutionary trends have been postulated by many authors from time to time on the basis of morphological features (WOMERSLEY and OPHEL, 1947; DESIKACHARY and SUNDARALINGAM, 1962; WOOD and IMAHORI, 1965) and cytological data (GUERLESQUIN, 1967; SARMA et al., 1970).

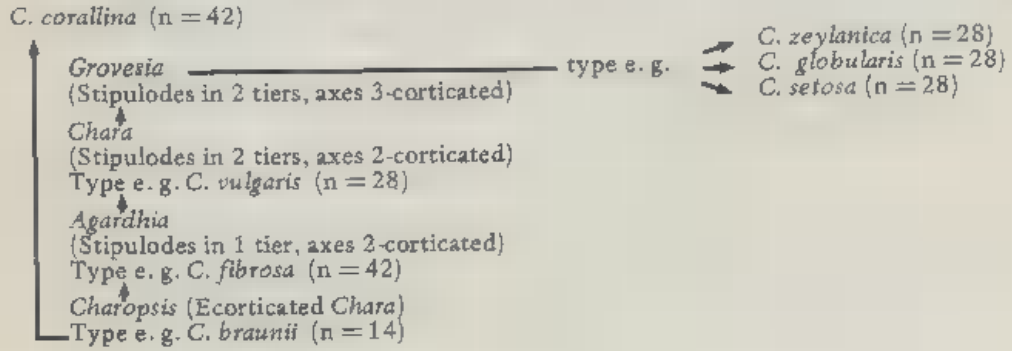
From the DNA data obtained in the present study an attempt has been made to correlate the DNA value of the respective species with regard to the phylogenetic interpretation of others.

According to the phylogenetic tree of SARMA et al. (1970), the Charopsis group is most primitive. Of the two species of this group studied *C. braunii* showed the least amount of DNA, but *C. corallina* of this group showed maximum amount of DNA. The nuclear volume and chromatin length were also maximum in this species. It implies that here the increase in DNA amount has been achieved through polyploidization. In the section *Charopsis*, the DNA content of *C. corallina* is at a higher level than the 42 or 28 chromosomes species belonging to *Agardhia*, *Chara* and *Grovesia*.

C. fibrosa belonging to *Agardhia* had 42 chromosomes, its nuclear volume and chromatin length were less in comparison to the other species.

C. vulgaris under the section *Chara* had DNA much less than the other 28 chromosomes species of *C. setosa*, *C. globularis* and *C. zeylanica* of the section *Grovesia*. Under the section *Grovesia* also variation in DNA content was observed. *C. zeylanica* showed the highest amount of DNA under this section.

Based on DNA amount data the following trends have been postulated :



ACKNOWLEDGEMENT : The authors thank Prof. A.K. SHARMA for laboratory facilities and to C.S.I.R., New Delhi for financial support.

REFERENCES

BENNETT M.D., 1972 — Nuclear DNA content and minimum generation time in herbaceous plants. *Proc. Roy. Soc. London* B. 181 : 109-135.

COLEMAN A.W., 1979 — Feulgen microspectrophotometric studies of *Pandorina morum* and other volvocales (Chlorophyceae). *J. Phycol.* 15 : 216-220.

DESIKACHARY T.V. and SUNDARALINGAM V.S., 1962 — Affinities and interrelationships of the Characeae. *Phycologia* 2 : 9-16.

GUERLESQUIN M., 1967 — Recherches caryotypiques et cytotaxinomiques chez les Charophycées d'Europe occidentale et d'Afrique du Nord. *Trav. Lab. Biol. Veg. et Phytogeogr. Fac. Lib. Sci. Angers* 22 : 1-265.

HOPKINS A.W., 1977 — Feulgen microspectrophotometric investigation of the life history of *Fritschiella tuberosa* (Chlorophyceae). *J. Phycol.* 13 : 321-323.

HULL H.M., HOSHAW R.W. and WANG C., 1982 — Cytofluorometric determination of nuclear DNA in living and preserved algae. *Stain Technol.* 57 : 273-282.

KOOP H.U., 1975 — Über den Ort der Meiose bei *Acetabularia mediterranea*. *Protoplasma* 85 : 109-114.

PAL R. and CHATTERJEE P., 1986 — Feulgen cytophotometric determination of nuclear DNA in species of *Nitella* (Charophyta) from India. *Cryptogamie, Algologie* 7 (2) : 129-134.

PRICE H.J., 1976 — Evolution of DNA content in higher plants. *Bot. Rev. (Lancaster)* 42 : 27-52.

SARMA Y.S.R.K., KHAN M. and RAMJEE, 1970 — A cytological approach to phylogeny, interrelationships and evolution in Charophyta. *Indian Biologist* 2 : 11-19.

SHEN E.Y.F., 1967 — Microspectrophotometric analysis of nuclear DNA in *Chara zeylanica*. *J. Cell Biol.* 35 (2 Part I) : 377-384.

STEBBINS G.L., 1950 — *Variation and Evolution in Plants*. New York, Columbia Univ. Press, Oxford Univ. Press, 643 p., 55 fig.

STEBBINS G.L., 1966 — Chromosome variation and evolution. *Science* 152 : 1463-1469.

WOMERSLEY H.B.S. and OPHEL I.L., 1947 — *Protochara*, a new genus of Characeae from Western Australia. *Trans. and Proc. Roy. Soc. South Australia* 71 : 311-317.

WOOD R.D. and IMAHORI K., 1964-65 — *A revision of the Characeae : Monograph of the Characeae*, Vol. 1, 1965, *Iconograph of the Characeae*, Vol. II, 1964, Weinheim, J. Cramer.